Prediction and assessment of retail hake (*Merluccius merluccius*) freshness during storage

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ABSTRACT

Reduction of fish freshness during the supply chain results into severe losses both from the economic (drastic reductions on market price) and environmental (fish rejection) points of view. In this context, prediction techniques based on mathematical models emerge as useful tools for fish quality assessment.
In this work we propose a methodology to forecast fish freshness during storage. In this regard, we found a strong correlation between fish freshness indicators commonly used in the industry, such as the Quality Sensory Method (QSM), and the growth of Specific Spoilage Organisms (SSO).
The main idea is to develop a reliable mathematical model which, on the one hand, describes the growth of SSO during storage and, on the other hand, relates the QSM to SSO concentration. Issues such as storage conditions, fishing practices or gutting procedures are taken into account. Such model allows us to predict shelf-life and quality losses.
In order to provide the model with reliable predictive capabilities, advanced model calibration techniques are employed. In this regard, optimal experimental design (OED) is used to generate sufficient non-isothermal informative experiments that allow us to reduce the uncertainty in the predictions. Also, the Monte Carlo sampling technique is exploited to detect the parameter confidence intervals and to calculate fish-to-fish variability in the predictions (core predictions).
The approach is illustrated using hake (*Merluccius merluccius*) caught either by bottom-set net or long-line under different storage temperature conditions. Furthermore, the effect of gutting is also studied. This methodology is flexible enough to consider other fish species or stress variables such as atmosphere composition.

Keywords: Fish shelf-life; Optimal experimental design; Fish freshness; Core predictions; Uncertainty analysis

INTRODUCTION

Fish quality assessment with sufficient accuracy and sensitivity is essential to set correct market prices, increase competitiveness, resolve conflicts of interest and prevent food wastage. Consequently, food researchers are now focusing in designing new mathematical tools able to monitor, predict and control fish quality (Dalgaard, 2002) using information from standard sensory, biochemical and microbiological experiments.
In this regard, classical approaches using statistical models (see for example Olafsdottir et al., 2006 and Carracosa et al., 2014) have proven to be fundamental to detect factors affecting shelf-life uncertainty, but of insufficient predictive capabilities to be exploited outside the set of experimental conditions. This limitation results crucial in real estimations of fish quality where storage conditions, such as temperature, are usually subject to fluctuations (dynamic changes).
Alternatively, food researchers have been exploring in the last decades the use of predictive dynamic models of microbial spoilage to estimate shelf-life under temperatures changes. Some examples are the models included in the software developed by Dalgaard et al. (2002), or the works by Koutsoumanis (2001) and Giuffrida et al. (2013) on seabream (*Sparus aurata*), Taoukis et al. (1999) on bogue (*Boops boops*) and Nuin et al. (2008) on fresh turbot (*Psetta maxima*).
However such models do not usually include the effect of fish-to-fish variability, use isothermal experiments to estimate model parameters (what may result into parameter estimates unable to reproduce temperature fluctuations) and fish shelf-life is assessed only in terms of time rejection, an indirect estimation of fish quality. In lasts years it should be stressed, however, that efforts have been conducted to overcome some of these limitations. See for example the work by Giuffrida et al. (2013) that exploits the potential of dynamic models to estimate freshness in terms of the Quality Index Method (QIM).

In this work, we make use of advance concepts in identification of dynamic models subject to parameter variability to predict different degrees of fish freshness in terms of the Quality Sensory Method (QSM) due to microbial changes in eviscerated hake captured by bottom-set nets. The methodology includes the design of optimal time-temperature profiles of temperature for accurate parameter estimation, calculation of parameter confidence intervals and evaluation of the prediction capabilities under fish-to-fish variability exploiting the Monte-Carlo method.
It should be stressed the ability of the method to predict the degree of freshness and its associated variability, as well as evaluate these changes due to differences in the fishing gear or handling protocol. In addition the methodology is flexible enough to include other stress variables ant to be extended to other fish species.

MATERIALS & METHODS

Fish handling, storage conditions, microbial analysis and sensorial analysis

Fresh gutted medium-sized hake (400-500 g) were purchased from the retail market during the first 24 h after slaughtering. Four specimens were analysed to assess initial quality and the boxes containing hake were sealed and stored under refrigeration conditions during 5-12 days. Gutted hake captured by bottom-set nets and stored at 1, 5 and 7 °C were used for calibration purposed. Gutted and un-gutted hake captured by bottom-set nets and long-line gear, stored at 3 °C were used for validation. Fish temperature was recorded every 5 min. Three to four specimens were taken out of refrigerated storage on a daily basis.
A quantity of 25 g of fish dorsal muscle was homogenized in 100 mL of 0.9\% NaCl. These homogenates were ten-fold serially diluted in peptone water. Aliquots (0.1 mL) of adequate dilutions were spread on glutamate starch phenol red agar (GSP) and Iron agar Lyngby (IAL). *Pseudomonas spp.* were counted as colonies grown on GSP plates after incubation at 25 °C during 48 h. Black colonies formed on IAL were counted after 3-5 days of incubation at 17 °C to enumerate H_2S-producing bacteria (*Shewanella spp.*).
Fish freshness was organoleptically assessed following the Quality Index Method (QIM) (Council Regulation (EC) No, 2406/96, 1996) developed by Baixas-Nogueras et al. (2003). Sensory quality was also assessed by reference to the freshness ratings for whitefish set out in Annex I of Council Regulation (EC) No 2406/96 (1996), named along the document as Quality Sensory Method (QSM).

Microbial spoilage model and numerical methods for model calibration

Pseudomonas and *Shewanella* are the two main bacterial groups responsible for spoilage of fresh fish (Gram & Dalgaard, 2002). These SSOs exhibit a growth and stationary phase following the standard logistic model (Baranyi & Roberts, 1994):

$$\frac{\mathrm{d}m}{\mathrm{d}t} = \mu(T)\left(1 - 10^{m - m^*}\right) \tag{1}$$

where m=*Ps*,*Sh* and m^*=*Ps**,*Sh** refer, respectively, to the concentration and maximum concentration of the selected microorganisms. The growth rate denoted as $\mu(T)$ depends on the temperature according to the standard square-root model (Ratkowsky et al., 1982):

$$\mu(T) = b(T - T^*) \tag{2}$$

With b=b_{ps} ,b_{sh} being the slope of the regression line and T^*=T_{ps}*,T_{sh}* an organism dependent reference temperature.

Unknown model parameters in equations (1)-(2) (namely, *Ps**,*Sh**, b_{ps} ,b_{sh}, T_{ps}*,T_{sh}*) are estimated using a one-step data regression that takes advantage of non-isothermal experiments to estimate all relevant parameters at once (Dolan et al., 2007). The underlying idea of the one-step model regression is to formulate an optimization problem where the objective is to compute those parameter values that minimize a measure of the distance among the model predictions and the experimental data. In this work this measure has been chosen to be the maximum likelihood (Walter & Pronzato, 1997).

The confidence on model parameters and predictions (an analysis known as core predictions) are evaluated using the Monte Carlo method. Optimal experimental design is also exploited to minimize the confidence intervals, and thus prediction uncertainty, with the minimum number of experiments and measurements.
For the sake of brevity, no further details are given about the parameter estimation problem, the Monte Carlo sampling method or optimal experimental design. The interested reader is referred to Walter & Pronzato (1997), Bernaerts et al. (2000), Balsa-Canto et al. (2008) and references therein.
To conclude this section, mention that the solution of the parameter estimation problem, the computation of confidence intervals and the optimal experimental design problem were solved using the AMIGO (Advanced Model Identification using Global Optimization) toolbox for Matlab™ (Balsa-Canto & Banga, 2011).

RESULTS & DISCUSSION

Parameter estimation

Solving equations (1)-(2) requires appropriate initial conditions which are affected by many factors such as fishing gear, handling, or catching ground and season. Inaccurate initial conditions may lead to over- or under-estimation of the growth curve and, therefore, they are included as parameters to be estimated.
Hake captured by bottom-set nets and stored at 1, 5 and 7 °C are used to perform an initial estimation. Confidence intervals for the estimated parameters were computed and used to compute the uncertainty in the model solutions by means of the *core predictions*. As shown in Figure 1(a) and (c), uncertainty on model parameters leads to a large uncertainty in model predictions.

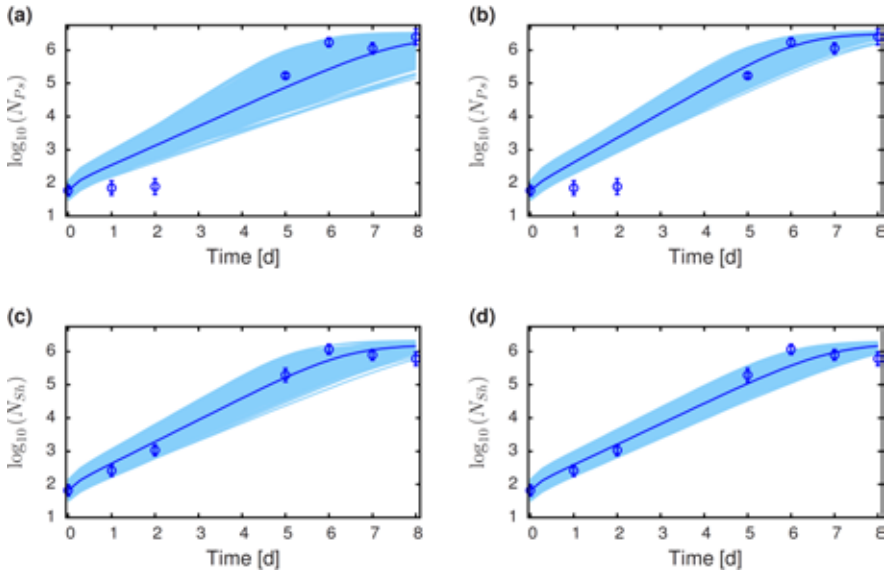

Figure 1. Comparison of core predictions in the validation experiment. **(a)** and **(c)** correspond with the results obtained using the initial preliminary experiments only, whereas **(b)** and **(d)** were estimated including the information of the Optimally Design experiment. Dots and bars represent experimental data, while continuous black lines show the most probable predictions. Bands correspond with the core predictions (uncertainty)

In order to improve the quality of the estimation, a new experiment was optimally designed. The duration of such experiment was fixed to 12 days. Since dynamic experiments are usually more informative, during such experiment one step in the storage temperature was allowed with a maximum of 11 °C and a minimum of 0 °C. The time at which the step takes place was also optimized. The solution of the OED problem led to an experiment that started with a storage temperature of 1 °C, at the eighth day such temperature changed to the maximum allowed (11 °C). The data of this experiment was used together with the other three to perform a new parameter estimation with the appropriate computation of the confidence intervals. Figure 1(b) and (d) show the core predictions (bands) after including the optimally designed experiment. As shown, uncertainty is largely reduced, in particular for *Pseudomonas*. For details regarding the parameter estimates and confidence intervals the reader is referred to García et al (2015).
It should be noted that Figure 1(a) and (b) suggest the presence of a lag time at least for low temperatures. Such lag time was not observed in experiments performed at higher temperatures (>3 °C). Baranyi &

Roberts (1994) proposed a way for including such lag time in the model equations. However, there are, at least, three reasons not to incorporate this modification in our model: first, the number of cells initially present in fish is low and the experimental error is rather important (more than 100 % in many cases) thus preventing the use of those data to estimate the lag time (Nuim et al., 2008); second, the model is intended to predict quality in the growth phase thus the lag will not have an impact on the final value; and third, the use of the modified model for the sake of prediction requires the knowledge about the physiological state of the cells at initial time, which is not available or measurable thus making the model useless.

It is important to mention that previous studies have reported a significant influence of the method of catching (Özyurt et al., 2007) and handling (Baixas-Nogueras et al., 2009) on microbial spoilage (or shelf-life) of fish. In order to test model predictive capabilities, gutted hake caught by a different fishing gear (long-line) and un-gutted hake caught by bottom-set nets were used to perform two new validation experiments. Results are shown in Figure 2(a) for long-line gear and Figure 2(b) for un-gutted hake. Despite some slight overestimation on the case of long-line gear, the model is able to reasonably predict the growth of *Pseudomonas.* Note that the influences of fishing gears and handling methods are embedded within the implicit uncertainty of the model. Similar results were obtained for *Shewanella*.

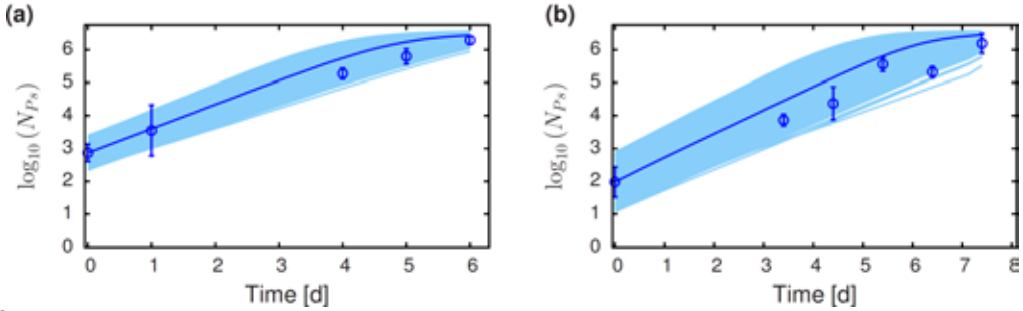

Figure 2. Comparison of core predictions in the validation experiment. A corresponds with the results for hake captured by long-line gear while B corresponds with un-gutted hake.

Quality predictions using the microbial spoilage model

In this section, a linkage between SSOs concentration and freshness indicators (QIM, QSM) will be stablished so that the predictive model developed above can be used to estimate fish shelf-life and quality. QIM classifies hake quality on a natural number scale which depends on the fish species. QSM establishes four categories: extra-quality (E); intermediate quality (A); intermediate quality (B) and not admitted (NA). In this study, we have found that SSO concentrations correlate particularly well with QIM via an expression of the form:

$$QIM = n_{int}\left(10^{IQ}\right) - 1; \qquad \text{where} \quad IQ = \alpha Ps + \beta Sh \tag{3}$$

where *QIM* is the corresponding quality index (ranging between 0-19 for hake (Baixas-Nogueras et al., 2003)), and n_{int} is the function that computes the nearest integer value. Data from the experiments used in the estimation, which included QIM and QSM evaluations from a panel of experts, have been used to estimate parameters in expression (3), resulting in $\alpha = 0.068$ and $\beta = 0.129$.

Figure 3 represents the most probable value of the QIM (stars) as well as its uncertainty (continuous blue lines) for the validation experiment represented in Figure 1. Such uncertainty was obtained by applying the results from the core predictions for microorganism concentrations *Sh* and *Ps* to equation (3). Values of the QSM and QIM from the panel of experts allowed us to stablish a connexion between the two indicators. Such connexion was used to draw the coloured regions in the background of Figure 3 defining the QSM grades. The existence of two overlapping categories was resolved by defining the new grades: A-B and B-NA.

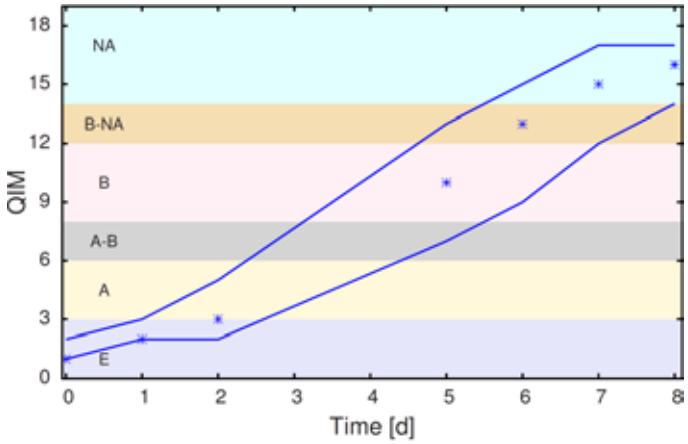

Figure 3. Most probable value of the QIM (stars) and its uncertainty (continuous lines) based on the microbial spoilage model. The colours in the background represent the QSM regions

The panel of experts evaluated the QSM in the experiment shown in Figure 3. Table 1 compares the values of QSM given by the panel of experts and the most probable value obtained by the predictive model. The range defined by QSM uncertainty is also given in the Table. Note that the marks obtained by the experts coincide for all times with the ones estimated by the model. It should be stressed that, despite the fact that the model here considered does not include the lag phase, quality predictions at the initial times coincide with the evaluations of the panel of experts. This supports the assumption that the effect of the latency time is not relevant to estimate quality.

Table 1. Values of the QSM estimated by the model and evaluated by the panel of experts

Time [days]	QSM	
	Model (range)	Sensory analysis, panel of experts
0	E (E-E)	E
1	E (E-A)	E
2	A (E-A)	A
5	B (B-B)	B
6	B-NA (B-NA)	B
7	NA (B-NA)	NA
8	NA (NA-NA)	NA

Shelf-life, understood as the time required to reach a not-admitted (NA) grade can be obtained in a straightforward manner from the quality prediction model. For the scenario depicted in Figure 3, a simple inspection gives a shelf-life range between approximately 5 to 8 days with a most probable value of 7 days, coinciding with the experimental evidence.

CONCLUSION

In this work we present a general methodology to develop sufficient informative models to predict fish quality and variability under changes of storage conditions. The approach was studied to find a growth model for specific spoilage microorganisms in hake (*Merluccius merluccius*) sufficiently informative to estimate quality sensory indexes under time-varying temperature profiles.

In addition, the model has been exploited to test the effect of factors such as the fishing gear or evisceration. Results from the microbial growth dynamics show no significant differences, within the inherent model uncertainty, between hake fished by long-line or bottom-set nets. Similar conclusions can be drawn for gutted and un-gutted hake along the experiment horizon.

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